

**567—41.2 (455B) Biological maximum contaminant levels (MCL) and monitoring requirements.****41.2(1) *Coliforms, fecal coliforms and E. coli.***

*a. Applicability.* These rules apply to all public water supply systems.  
*b. Maximum contaminant levels (MCL) for total coliforms, fecal coliforms, and E. coli.* The MCL is based on the presence or absence of total coliforms in a sample.

**(1) Nonacute coliform bacteria MCL.**

1. For a system which collects 40 samples or more per month, no more than 5.0 percent of the samples collected during a month may be total coliform-positive. A nonacute total coliform bacteria MCL violation occurs when more than 5.0 percent of routine and repeat samples collected during a month are total coliform-positive, but are not fecal coliform-positive or *E. coli*-positive.

2. For a system which collects less than 40 samples per month, no more than one sample collected during a month may be total coliform-positive. A nonacute total coliform bacteria MCL violation occurs when two or more routine and repeat samples collected during a month are total coliform-positive, but are not fecal coliform-positive or *E. coli*-positive.

(2) Acute coliform bacteria MCL. Any fecal coliform-positive repeat sample or *E. coli*-positive repeat sample, or any total coliform-positive repeat sample following a fecal coliform-positive or *E. coli*-positive routine sample constitutes a violation of the MCL for total coliforms. For purposes of the public notification requirements in 567—42.1(455B), this is a violation that may pose an acute risk to health.

(3) MCL compliance period. Compliance of a system with the MCL for total coliforms in 41.2(1)“b”(1) and (2) is based on each month in which the system is required to monitor for total coliforms.

(4) Compliance determination. Results of all routine and repeat samples not invalidated by the department or laboratory must be included in determining compliance with the MCL for total coliforms. Repeat samples must be analyzed at the same laboratory as the corresponding original routine sample(s), unless written approval for use of a different laboratory is granted by the department.

*c. Monitoring requirements.***(1) Routine total coliform monitoring.**

1. Public water supply systems must collect total coliform samples at sites which are representative of water throughout the distribution system according to a written sample siting plan. The plan shall be reviewed or updated by the public water supply system every two years and shall be retained on file at the facility. Major elements of the plan shall include, but are not limited to, a map of the distribution system, notation or a list of routine sample location(s) for each sample period, resample locations for each routine sample, and a log of samples taken. The plan must be made available to the department upon request and during sanitary surveys and must be revised by the system as directed by the department.

2. The public water supply system must collect samples at regular time intervals throughout the month, except that a system which uses only groundwater that is not under the direct influence of surface water and which is required to collect five or fewer routine coliform bacteria samples per month may collect all required samples on a single day if they are taken from different sites. A system that uses only groundwater and adds a chemical disinfectant or provides water with a disinfectant must measure the residual disinfectant concentration at the same points in the distribution system and at the same time as total coliform bacteria samples are collected. A system that uses surface water or IGW must comply with the requirements specified in 567—paragraph 43.5(4)“b”(2)“2.” The system shall report the residual disinfectant concentration to the laboratory with the bacteria sample and comply with the applicable reporting requirements of 567—subrule 42.4(3).

3. Community water systems and specific noncommunity systems. The monitoring frequency for total coliforms for community water systems and noncommunity water systems serving schools, to include preschools and child care facilities or serving public water systems owned or managed by state

agencies, such as state parks and rest areas, is based on the population served by the system as listed below, until June 29, 1994. Public water systems which do not collect five or more routine samples each month must undergo an initial sanitary survey by June 29, 1994. After June 29, 1994, the monitoring frequency for systems serving less than 4,101 persons shall be a minimum of five routine samples per month unless the department determines, after completing sanitary surveys (at intervals not to exceed five years), that the monitoring frequency may continue as listed below. The monitoring frequency for regional water systems shall be as listed in 41.2(1)“c”(1)“4” but in no instance less than that required by the population equivalent served.

TOTAL COLIFORM MONITORING FREQUENCY FOR COMMUNITY  
WATER SYSTEMS AND NONCOMMUNITY (SCHOOL) WATER SYSTEMS

<u>Population Served</u>	<u>Minimum Number of Samples Per Month</u>
25 to 1,000*	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 12,900	10
12,901 to 17,200	15
17,201 to 21,500	20
21,501 to 25,000	25
25,001 to 33,000	30
33,001 to 41,000	40
41,001 to 50,000	50
50,001 to 59,000	60
59,001 to 70,000	70
70,001 to 83,000	80
83,001 to 96,000	90

96,001 to 130,000	100
130,001 to 220,000	120
220,001 to 320,000	150
320,001 to 450,000	180
450,001 to 600,000	210
600,001 to 780,000	240
780,001 to 970,000	270

\*Includes public water supply systems which have at least 15 service connections, but serve fewer than 25 persons

4. Regional water systems. The supplier of water for a regional water system as defined in rule 567—40.2(455B) shall sample for coliform bacteria at a frequency indicated in the following chart until June 29, 1994, but in no case shall the sampling frequency for a regional water system be less than as set forth in 41.2(1)“c”(1)“3” based on the population equivalent served. Public water systems which do not collect five or more routine samples each month must undergo an initial sanitary survey by June 29, 1994. After June 29, 1994, the monitoring frequency of systems with less than 82 miles of pipe shall be a minimum of five routine samples per month unless the department determines, after completing sanitary surveys (at intervals not exceeding five years), that the monitoring frequency may continue as listed below. The following chart represents sampling frequency per miles of distribution system and is determined by calculating one-half the square root of the miles of pipe.

TOTAL COLIFORM MONITORING FREQUENCY FOR  
REGIONAL WATER SYSTEMS

<u>Miles of Pipe</u>	<u>Minimum Number of Samples Per Month</u>
0 - 9	1
10 - 25	2
26 - 49	3
50 - 81	4
82 - 121	5
122 - 169	6
170 - 225	7
226 - 289	8
290 - 361	9
362 - 441	10

442 - 529	11
530 - 625	12
626 - 729	13
730 - 841	14
842 - 961	15
962 - 1,089	16
1,090 - 1,225	17
1,226 - 1,364	18
1,365 - 1,521	19
1,522 - 1,681	20
1,682 - 1,849	21
1,850 - 2,025	22
2,026 - 2,209	23
2,210 - 2,401	24
2,402 - 2,601	25
2,602 - 3,249	28
3,250 - 3,721	30
3,722 - 4,489	33
greater than 4,489	35

5. Noncommunity water systems. The monitoring frequency for total coliforms for noncommunity water systems is as listed in the four unnumbered paragraphs below until June 29, 1999. Public water systems which do not collect five or more routine samples each month must undergo an initial sanitary survey by June 29, 1999. After June 29, 1999, the minimum number of samples shall be five routine samples per month unless the department determines, after completing sanitary surveys (at intervals not exceeding five years), that the monitoring frequency may continue as listed below. A noncommunity water system using only groundwater (except groundwater under the direct influence of surface water, as defined in 567—paragraph 43.5(1)“b”) and serving 1,000 persons or fewer must monitor each calendar quarter that the system provides water to the public. Systems serving more than 1,000 persons during any month must monitor at the same frequency as a like-sized community water system, as specified in 41.2(1)“c”(1)“3.”

A noncommunity water system using surface water, in total or in part, must monitor at the same frequency as a like-sized community water system, as specified in 41.2(1)“c”(1)“3,” regardless of the number of persons it serves.

A noncommunity water system using groundwater under the direct influence of surface water, as defined in 567—paragraph 43.5(1) “b,” must monitor at the same frequency as a like-sized community water system, as specified in 41.2(1) “c”(1)“3,” regardless of the number of persons it serves. The system must begin monitoring at this frequency beginning six months after the department determines that the groundwater is under the direct influence of surface water.

A noncommunity water system serving schools or daycares must monitor at the same frequency as a like-sized community water system, as specified in 41.2(1) “c”(1)“3.”

A noncommunity water system owned or managed by a state agency, such as a park or rest area, must monitor at the same frequency as a like-sized community water system, as specified in 41.2(1) “c”(1)“3.”

6. If the department, on the basis of a sanitary survey or monitoring results history, determines that some greater frequency of monitoring is more appropriate, that frequency shall be the frequency required under these regulations. This frequency shall be confirmed or changed on the basis of subsequent surveys.

7. Special purpose samples, such as those taken to determine whether disinfection practices are sufficient following pipe placement, replacement, or repair, shall not be used to determine compliance with the MCL for total coliforms in 41.2(1) “b.” Repeat samples taken pursuant to 41.2(1) “c”(2) are not considered special purpose samples and must be used to determine compliance with the MCL for total coliforms in 41.2(1) “b.”

(2) Repeat total coliform monitoring.

1. Repeat sample time limit and numbers. If a routine sample is total coliform-positive, the public water supply system must collect a set of repeat samples within 24 hours of being notified of the positive result and in no case more than 24 hours after being notified by the department. A system which collects more than one routine sample per month must collect no fewer than three repeat samples for each total coliform-positive sample found. A system which collects one routine sample per month or fewer must collect no fewer than four repeat samples for each total coliform-positive sample found. The department may extend the 24-hour limit on a case-by-case basis if the system has a logistical problem in collecting the repeat samples within 24 hours that is beyond its control. In those cases, the public water supply system must report the circumstances to the department no later than the end of the next business day after receiving the notice to repeat sample and initiate the action directed by the department. In the case of an extension, the department will specify how much time the system has to collect the repeat samples.

2. Repeat sample location(s). The system must collect at least one repeat sample from the sampling tap where the original total coliform-positive sample was taken, at least one repeat sample at a tap within five service connections upstream and at least one repeat sample at a tap within five service connections downstream of the original sampling site. If a total coliform-positive sample is at the end of the distribution system, or at the first or last service connection, the system will be required to collect the repeat samples from the original sampling site and locations only upstream or downstream.

3. The system must collect all repeat samples on the same day, except that the department may allow a system with a single service connection to collect the required set of repeat samples over a four-day period. “System with a single service connection” means a system which supplies drinking water to consumers through a single service line.

4. Additional repeat sampling. If one or more repeat samples in the set is total coliform-positive, the public water supply system must collect an additional set of repeat samples in the manner specified in 41.2(1) “c”(2)“1” to 41.2(1) “c”(2)“3.” The system must repeat this process until either total coliforms are not detected in one complete set of repeat samples or the system determines that the MCL for total coliforms in 41.2(1) “b” has been exceeded, notifies the department, and provides public notification to its users in accordance with 567—42.1(455B).

5. If a system collecting fewer than five routine samples per month has one or more total coliform-positive samples and the department does not invalidate the sample(s) under 41.2(1) “c”(3), it must collect at least five routine samples during the next month the system provides water to the public.

For systems monitoring on a quarterly basis, the additional five routine samples may be required to be taken within the same quarter in which the original total coliform-positive sample occurred.

The department may waive the requirement to collect five routine samples the next month the system provides water to the public if the department has determined through an on-site visit the reason that the sample was total coliform-positive and establishes that the system has corrected the problem or will correct the problem before the end of the next month the system serves water to the public. In this case, the department must document this decision to waive the following month's additional monitoring requirement in writing, have it approved and signed by the supervisor of the water supply section and the department official who recommends such a decision, and make this document available to the EPA and public. The written documentation will generally be provided by the public water supply system in the form of a request and must describe the specific cause of the total coliform-positive sample and what action the system has taken to correct the problem. The department will not waive the requirement to collect five routine samples the next month the system provides water to the public solely on the grounds that all repeat samples are total coliform-negative. If the requirement to collect five routine samples is waived under this paragraph, a system must still take at least one routine sample before the end of the next month it serves water to the public and use it to determine compliance with the MCL for total coliforms in 41.2(1) "b."

(3) Invalidation of total coliform samples. A total coliform-positive sample invalidated under this subparagraph does not count towards meeting the minimum monitoring requirements of 41.2(1) "c." The department may invalidate a total coliform-positive sample only if one or more of the following conditions are met.

1. The laboratory establishes that improper sample analysis caused the total coliform-positive result. A laboratory must invalidate a total coliform sample (unless total coliforms are detected, in which case, the sample is valid) if the sample produces a turbid culture in the absence of gas production using an analytical method where gas formation is examined (e.g., the multiple tube fermentation technique), produces a turbid culture in the absence of an acid reaction in the presence-absence (P-A) coliform test, or exhibits confluent growth or produces colonies too numerous to count with an analytical method using a membrane filter (e.g., membrane filter technique). If a laboratory invalidates a sample because of such interference, the system must collect another sample from the same location as the original sample within 24 hours of being notified of the interference problem, and have it analyzed for the presence of total coliforms. The system must continue to resample within and have the samples analyzed until it obtains a valid result. The department may waive the 24-hour time limit on a case-by-case basis.

2. The department, on the basis of the results of repeat samples collected as required by 41.2(1) "c"(2) "1" to "4," determines that the total coliform-positive sample resulted from a domestic or other nondistribution system plumbing problem. "Domestic or other nondistribution system plumbing problem" means a coliform contamination problem in a public water supply system with more than one service connection that is limited to the specific service connection from which the coliform-positive sample was taken. The department will not invalidate a sample on the basis of repeat sample results unless all repeat samples collected at the same tap as the original total coliform-positive sample are also total coliform-positive, and all repeat samples collected within five service connections of the original tap are total coliform-negative (e.g., the department will not invalidate a total coliform-positive sample on the basis of repeat samples if all the repeat samples are total coliform-negative, or if the public water supply system has only one service connection).

3. The department has substantial grounds to believe that a total coliform-positive result is due to a circumstance or condition which does not reflect water quality in the distribution system. In this case, the system must still collect all repeat samples required under 41.2(1) "c"(2) "1" to "4," and use them to determine compliance with the MCL for total coliforms in 41.2(1) "b." To invalidate a total coliform-positive sample under this paragraph, the decision with the rationale for the decision must be documented in writing and approved and signed by the supervisor of the water supply section and the department official who recommended the decision. The department must make this document available to EPA and the public. The written documentation generally provided by the public water

supply system in the form of a request must state the specific cause of the total coliform-positive sample, and what action the system has taken to correct this problem. The department will not invalidate a total coliform-positive sample solely on the grounds of poor sampling technique or that all repeat samples are total coliform-negative.

(4) Fecal coliforms/*Escherichia coli* (*E. coli*) testing.

1. If any routine or repeat sample is total coliform-positive, the system must analyze that total coliform-positive culture medium to determine if fecal coliforms are present, except that the system may test for *E. coli* in lieu of fecal coliforms.

2. The department may allow a public water supply system, on a case-by-case basis, to forego fecal coliform or *E. coli* on a total coliform-positive sample if that system assumes that the total coliform-positive sample is fecal coliform-positive or *E. coli*-positive. Accordingly, the system must notify the department as specified in 41.2(1) “c”(5) “1” and meet the provisions of 567—42.1(455B) pertaining to public notification.

(5) Public water supply system’s response to violation.

1. A public water supply system which has exceeded the MCL for total coliforms in 41.2(1) “b” must report the violation to the water supply section of the department by telephone no later than the end of the next business day after it learns of the violation, and notify the public in accordance with 567—42.1(455B).

2. A public water supply system which has failed to comply with a coliform monitoring requirement must report the monitoring violation to the department within ten days after the system discovers the violation and notify the public in accordance with 567—42.1(455B).

3. If fecal coliforms or *E. coli* are detected in a routine or repeat sample, the system must notify the department by telephone by the end of the day when the system is notified of the test result, unless the system is notified of the result after the department office is closed, in which case the system must notify the department before the end of the next business day. If the detection of fecal coliform or *E. coli* in a sample causes a violation of the MCL, the system is required to notify the public in accordance with 567—42.1(455B).

*d. Best available technology (BAT).* The U.S. EPA identifies, and the department has adopted, the following as the best technology, treatment techniques, or other means available for achieving compliance with the maximum contaminant level for total coliforms in 41.2(1) “b.”

(1) Well protection. Protection of wells from contamination by coliforms by appropriate placement and construction;

(2) Disinfectant residual. Maintenance of a disinfectant residual throughout the distribution system;

(3) Distribution system maintenance. Proper maintenance of the distribution system including appropriate pipe replacement and repair procedures, main flushing programs, proper operation and maintenance of storage tanks and reservoirs, and continual maintenance of a minimum positive water pressure of 20 psig in all parts of the distribution system at all times; and

(4) Filtration or disinfection. Filtration and disinfection of surface water or groundwater under the direct influence of surface water in accordance with 567—43.5(455B) or disinfection of groundwater using strong oxidants such as, but not limited to, chlorine, chlorine dioxide, or ozone.

(5) Wellhead protection program. For groundwater systems, compliance with the requirements of the department’s wellhead protection program.

*e. Analytical methodology.*

(1) Sample volume. The standard sample volume required for total coliform analysis, regardless of analytical method used, is 100 mL.

(2) Presence/absence determination. Public water supply systems shall determine the presence or absence of total coliforms. A determination of total coliform density is not required.

(3) Total coliform bacteria analytical methodology. Public water supply systems must conduct total coliform analyses in accordance with one of the analytical methods in the following table:

Organism	Methodology <sup>12</sup>	Citation <sup>1</sup>
Total Coliforms <sup>2</sup>	Total Coliform Fermentation Technique <sup>3,4,5</sup>	9221A, B
	Total Coliform Membrane Filter Technique <sup>6</sup>	9222A, B, C
	Presence-Absence (P-A) Coliform Test <sup>5,7</sup>	9221D
	ONPG-MUG Test <sup>8</sup>	9223
	Colisure Test <sup>9</sup>	
	E*Colite Test <sup>10</sup>	
	m-ColiBlue24 Test <sup>11</sup>	
	ReadyCult Coliforms 100 Presence/Absence Test <sup>13</sup>	
	Membrane Filter Technique Using Chromocult Coliform Agar <sup>14</sup>	
	Colitag Test <sup>15</sup>	

The procedures shall be done in accordance with the documents listed below. The incorporation by reference of the following documents listed in footnotes 1, 6, 8, 9, 10, 11, 13, 14, and 15 was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies of the documents may be obtained from the sources listed below. Information regarding obtaining these documents can be obtained from the Safe Drinking Water Hotline at (800)426-4791. Documents may be inspected at EPA's Drinking Water Docket, EPA West, 1301 Constitution Avenue NW, Room B102, Washington, DC 20460, telephone (202)566-2426; or at the Office of Federal Register, 800 North Capitol Street NW, Suite 700, Washington, DC 20408.

<sup>1</sup>Methods 9221A, B; 9222A, B, C; 9221D; and 9223 are contained in Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, 19th edition, 1995, or 20th edition, 1998, American Public Health Association, 1015 Fifteenth Street NW, Washington, DC 20005. The cited methods published in any of these three editions may be used.

<sup>2</sup>The time from sample collection to initiation of the analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10 degrees Celsius during transit.

<sup>3</sup>Lactose broth, as commercially available, may be used in lieu of lauryl tryptose broth, if the system conducts at least 25 parallel tests between this medium and lauryl tryptose broth using the water normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliforms, using lactose broth, is less than 10 percent.

<sup>4</sup>If inverted tubes are used to detect gas production, the media should cover these tubes at least one-half to two-thirds after the sample is added.

<sup>5</sup>No requirement exists to run the completed phase on 10 percent of all total coliform-positive confirmed tubes.

<sup>6</sup>MI agar also may be used. Preparation and use of MI agar is set forth in the article, "New medium for the simultaneous detection of total coliform and *Escherichia coli* in water," by Brenner, K.P., et al., 1993, Applied Environmental Microbiology 59:3534-3544. Also available from the Office of Water Resource Center (RC-4100), 401 M Street SW, Washington, DC 20460, EPA 600/J-99/225.

<sup>7</sup>Six-times formulation strength may be used if the medium is filter-sterilized rather than autoclaved.

<sup>8</sup>The ONPG-MUG Test is also known as the Autoanalysis Colilert System.



<sup>9</sup>The Colisure Test may be read after an incubation time of 24 hours. A description of the Colisure Test, February 28, 1994, may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092.

<sup>10</sup>A description of the E\*Colite Test, "Presence/Absence for Coliforms and *E. coli* in Water," December 21, 1997, is available from Charm Sciences, Inc., 25 Franklin Street, Malden, MA 02148-4120.

<sup>11</sup>A description of the m-ColiBlue24 Test, August 17, 1999, is available from the Hach Company, 100 Dayton Avenue, Ames, IA 50010.

<sup>12</sup>The department strongly recommends that laboratories evaluate the false-positive and false-negative rates for the method(s) they use for monitoring total coliforms. It also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method chosen has an unacceptable false-positive or false-negative rate, another method may be used. The department suggests that laboratories perform these studies on a minimum of 5 percent of all total coliform-positive samples, except for those methods for which verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and false-negative rates may be based on lactose fermentation, the rapid test for beta-galactosidase and cytochrome oxidase, multitest identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies or from the manufacturer(s).

<sup>13</sup>The ReadyCult Coliforms 100 Presence/Absence Test is described in the document, "ReadyCult Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," November 2000, Version 1.0, available from EM Science, 480 S. Democrat Road, Gibbstown, NJ 08027-1297, telephone: (800)222-0342, E-mail address: [adellenbusch@emscience.com](mailto:adellenbusch@emscience.com).

<sup>14</sup>Membrane Filter Technique using Chromocult Coliform Agar is described in the document, "Chromocult Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," November 2000, Version 1.0, available from EM Science, 480 S. Democrat Road, Gibbstown, NJ 08027-1297, telephone: (800)222-0342, E-mail address: [adellenbusch@emscience.com](mailto:adellenbusch@emscience.com).

<sup>15</sup>Colitag product for the determination of the presence/absence of total coliforms and *E. coli* is described in "Colitag Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations," August 2001, available from CPI International, Inc., 5580 Skylane Blvd., Santa Rosa, CA 95403, telephone: (800)878-7654, Internet address: [www.cpiinternational.com](http://www.cpiinternational.com).

(4) Rescinded IAB 8/11/99, effective 9/15/99.

(5) Fecal coliform analytical methodology. Public water systems must conduct fecal coliform analysis in accordance with the following procedure. When the MTF Technique or presence-absence (P-A) coliform test is used to test for total coliforms, shake the lactose-positive presumptive tube or P-A bottle vigorously and transfer the growth with a sterile 3-mm loop or sterile applicator stick into brilliant green lactose bile broth and EC medium to determine the presence of total and fecal coliforms, respectively. For EPA-approved analytical methods which use a membrane filter, transfer the total coliform-positive culture by one of the following methods: remove the membrane containing the total coliform colonies from the substrate with sterile forceps and carefully curl and insert the membrane into a tube of EC medium (the laboratory may first remove a small portion of selected colonies for verification); swab the entire membrane filter surface with a sterile cotton swab and transfer the inoculum to EC medium (do not leave the cotton swab in the EC medium); or inoculate individual total coliform-positive colonies into EC medium. Gently shake the inoculated EC tubes to ensure adequate mixing and incubate in a waterbath at 44.5 ( + or - ) 0.2 degrees C for 24 ( + or - ) 2 hours. Gas production of any amount in the inner fermentation tube of the EC medium indicates a positive fecal coliform test. The preparation of EC medium is described in Method 9221E (paragraph 1a) in Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, 19th edition, 1995, and 20th edition, 1998; the cited method in any one of these three editions may be used. Public water supply systems need only determine the presence or absence of fecal coliforms; a determination of fecal coliform density is not required.

(6) *E. coli* analytical methodology. Public water systems must conduct analysis of *Escherichia coli* (*E. coli*) in accordance with one of the following analytical methods:

1. EC medium supplemented with 50 micrograms per milliliter of 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration), as described in Method 9222G

in Standard Methods for the Examination of Water and Wastewater, 19th edition (1995) and 20th edition (1998). Either edition may be used. Alternatively, the 18th edition (1992) may be used if at least 10 mL of EC medium, as previously described in subparagraph 41.2(1)“e”(5), is supplemented with 50 micrograms/mL of MUG before autoclaving. The inner inverted fermentation tube may be omitted. If the 18th edition is used, apply the procedure in subparagraph 41.2(1)“e”(5) for transferring a total coliform-positive culture to EC medium supplemented with MUG, incubate the tube at 44.5 plus or minus 0.2 degrees Celsius for 24 plus or minus 2 hours, and then observe fluorescence with an ultraviolet light (366 nm) in the dark. If fluorescence is visible, *E. coli* are present.

2. Nutrient agar supplemented with 100 micrograms per mL 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration). Nutrient agar is described in Method 9222G in Standard Methods for the Examination of Water and Wastewater, 19th edition (1995) and 20th edition (1998). Either edition may be used for determining if a total coliform-positive sample, as determined by a membrane filter technique, contains *E. coli*. Alternatively, the 18th edition (1992) may be used if the membrane filter containing a total coliform-positive colony(ies) is transferred to nutrient agar, as described in Method 9221B (paragraph 3) of Standard Methods (18th edition), supplemented with 100 micrograms/mL of MUG. If the 18th edition is used, incubate the agar plate at 35 degrees Celsius for 4 hours and then observe the colony(ies) under ultraviolet light (366 nm) in the dark for fluorescence. If fluorescence is visible, *E. coli* is present.

3. Minimal Medium ONPG-MUG (MMO-MUG) Test, as set forth in the article “National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparisons with Presence-Absence Techniques” (Edberg et al.), Applied and Environmental Microbiology, Volume 55, pp. 1003-1008, April 1989. (Note: The Autoanalysis Colilert System is an MMO-MUG test.) If the MMO-MUG Test is total coliform-positive after a 24-hour incubation, test the medium for fluorescence with a 366-nm ultraviolet light (preferably with a 6-watt lamp) in the dark. If fluorescence is observed, the sample is *E. coli*-positive. If fluorescence is questionable (cannot be definitively read) after 24 hours incubation, incubate the culture for an additional 4 hours (but not to exceed 28 hours total), and again test the medium for fluorescence. The MMO-MUG Test with hepes buffer is the only approved formulation for the detection of *E. coli*.

4. The membrane filter method with MI agar, as described in footnote 6 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

5. E\*Colite Test, as described in footnote 10 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

6. m-ColiBlue 24 Test, as described in footnote 11 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

7. Colisure Test, as described in footnote 9 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

8. Readycult Coliforms 100 Presence/Absence Test, as described in footnote 13 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

9. Membrane Filter Technique using Chromocult Coliform Agar, as described in footnote 14 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

10. Colitag, as described in footnote 15 of the Total Coliform Methodology Table in 41.2(1)“e”(3).

(7) Optional *E. coli* analytical methodology. As an option to 41.2(1)“e”(6) a system with a total coliform-positive, MUG-negative, MMO-MUG Test may further analyze the culture for the presence of *E. coli* by transferring a 0.1 mL, 28-hour MMO-MUG culture to EC Medium + MUG with a pipette. The formulation and incubation conditions of EC Medium + MUG and observation of the results are described in 41.2(1)“e”(6).

**41.2(2)** *Giardia*. Reserved.

**41.2(3)** *Heterotrophic plate count bacteria (HPC)*.

a. *Applicability*. All public water systems that use a surface water source or source under the direct influence of surface water must provide treatment consisting of disinfection, as specified

in 567—subrule 43.5(2), and filtration treatment which complies with 567—subrule 43.5(3). The heterotrophic plate count is an alternate method to demonstrate a detectable disinfectant residual in accordance with 567—paragraph 43.5(2) “d.”

- b. Maximum contaminant levels.* Reserved.
- c. Monitoring requirements.* Reserved.
- d. BAT.* Reserved.

*e. Analytical methodology.* Public water systems shall conduct heterotrophic plate count bacteria analysis in accordance with 567—subrule 43.5(2) and the following analytical method. Measurements for heterotrophic plate count bacteria must be conducted by a laboratory certified by the department to do such analysis, when heterotrophic plate count bacteria are being measured in lieu of a detectable residual disinfectant pursuant to 567—paragraph 43.5(2) “d.” In addition, the time from sample collection to initiation of analysis may not exceed eight hours, and the systems must hold the samples below 10 degrees Celsius during transit to the laboratory.

(1) Method. The heterotrophic plate count shall be performed in accordance with one of the following methods:

1. Method 9215B Pour Plate Method, Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, 19th edition, 1995, or 20th edition, 1998. The cited method in any of the three editions may be used.

2. SimPlate Method, “IDEXX SimPlate TM HPC Test Method for Heterotrophs in Water,” November 2000, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092, telephone (800)321-0207.

(2) Reporting. The public water system shall report the results of heterotrophic plate count in accordance with 567—subparagraph 42.4(3) “c”(2).

#### **41.2(4) Macroscopic organisms and algae.**

*a. Applicability.* These rules apply to both community and noncommunity public water supply systems using surface water or groundwater under direct influence of surface water as defined by 567—subrule 43.5(1).

*b. Maximum contaminant levels (MCLs) for macroscopic organisms and algae.* Finished water shall be free of any macroscopic organisms such as plankton, worms, or cysts. The finished water algal cell count shall not exceed 500 organisms per milliliter or 10 percent of the total cells found in the raw water, whichever is greater.

- c. Monitoring requirements.* Reserved.
- d. BAT.* Reserved.

*e. Analytical methodology.* Measurement of the algal cells shall be in accordance with Method 10200F: Phytoplankton Counting Techniques, Standard Methods for the Examination of Water and Wastewater, 18th edition, pp. 10-13 to 10-16. Such measurement shall be required only when the department determines on the basis of complaints or otherwise that excessive algal cells may be present.